THE EFFECT OF INHIBITION OF NON-SPECIFIC CHOLINESTERASE ON PERCEPTION OF TACTILE SENSATION IN HUMAN VOLAR SKIN*

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Recently it has been demonstrated that high concentrations of non-specific (pseudo-, butyro-) cholinesterase (ChE) are present in some of the specialized sensory end-organs of human skin. The enzyme was first visualized in the tactile corpuscles of Meissner (1) and later in Pacinian corpuscles (2). Both these endings are found in abundance in the skin of the palms and soles. The former are believed to subserve the sensation of light touch and the latter that of pressure in volar skin and in other areas in which they are found. In addition the enzyme has also been demonstrated in genital corpuscles (3).

The precise function of non-specific ChE is unknown. Its diverse distribution (plasma, hepatic cells; lining cells, interstitial cells, smooth muscle and occasional ganglion cells of the small intestine; groups of chemoreceptor cells in the carotid body; capsular glial cells of sensory and autonomic ganglia; Schwann's sheath cells; glial cells of the white and gray fiber tracts of the central nervous system; certain sensory endings as mentioned above) would seem to indicate a non-specific biochemical role. Unlike specific (true, aceto-, acetyl-) ChE, which is of primary importance in neuromuscular transmission and in the transmission of cholinergic impulses both centrally and at peripheral neuro-effector junctions, non-specific ChE is believed not to be concerned with the mediation of nervous impulses. In some locations, as in autonomic ganglia, for example, it has been suggested that it may act in a supportive role, augmenting the specific ChE activity of this site. In sensory ganglia, non-specific ChE may prevent acetylcholine liberated elsewhere from affecting these neurons (4). There is little experimental evidence to support these speculations, however, and we are still unable to assign to this enzyme any function of significance in the transmission or reception of nervous impulses. Indeed, its physiological substrate may not be acetylcholine (ACh).

The recent data indicating the presence of non-specific ChE in high concentrations in specialized sensory nerve-endings in the skin suggested that this enzyme might play a role in the perception of cutaneous sensation. In an effort to examine further this possibility, we have studied the effects on certain forms of cutaneous sensation of inhibition of ChE's by locally applied diisopropylfluorophosphate (DFP). Inhibition of ChE's in the treated skin was confirmed by histochemical studies. Volar skin was chosen as the test area because of the preponderance of specialized and non-specialized nerve-endings found there.

EXPERIMENTAL

The volar skin of the great toes of four healthy adult, white males was cleansed with soap and water and rinsed with acetone. The perception of light touch, sharp-dull discrimination, pressure and pricking pain was tested as follows:

1. Lightly stroking area with a coarse woolen cloth.
2. Light application of the sharp or blunt end of a pin.
3. Moderately firm (enough to indent skin slightly) application of the blunt end of a pin.
4. Firm application of the sharp end of a #23 needle.

It was determined that in each subject the perception of these sensations was roughly equivalent in each great toe and within the normal range of sensitivity as compared to other normal adult males.

0.02 cc. of distilled water was then pipetted on to the volar skin of one great toe within a round area 1.3 cm. in diameter, and 0.02 cc. of undiluted DFP was placed on a similar area of equal size on the opposite great toe. Both areas were quickly

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capped with a small glass cup (leaving a small air space 0.5 cm. in height above the skin) which was taped firmly to the surrounding skin. In view of the potency and volatility of DFP, considerable care was taken to prevent contamination of skin specimens, even at a distance. In this regard, we routinely applied (and capped) the control (distilled water) area prior to the DFP in each subject.

Both liquids were allowed to remain in this manner for one hour. Blood pressure and pulse rate were recorded at the outset, every ten minutes during the application of the cups and at one hour following their removal as a precaution against possible development of systemic intoxication. The test skin areas were scrutinized for changes in skin color and sweat gland activity. Following the removal of the cups the treated skin was re-examined for sensory perception in the same manner as before. On two of the subjects, six mm. punch biopsy specimens were then taken under procaine (2%) digital block anesthesia. The skin specimens secured were placed on dry ice and taken to the laboratory where they were cut as frozen sections at 10–15 microns and carried through the histochemical procedure of Koelle for the localization of cholinesterases, employing substrates and inhibitors as described most recently (5). Incubation periods were one, two and four hours.

OBSERVATIONS

Upon removal of the glass cups, grossly visible eccrine sweat droplets were evident on the skin of the DFP-treated toes of all four subjects studied. The skin of the control, water-treated toes showed no such secretion, however, nor did any other skin areas of either foot. There was no color change of the test skin of either the DFP-treated or control toes and there was no discernible alteration in the skin temperature at these sites. Examination of the toes at this time for sensory perception by the aforementioned tests failed to reveal any significant differences between the control and DFP-treated toes, and there was no variation from the initial pre-treatment evaluation.

Histochemically, the volar skin treated with distilled water showed the previously reported localization of specific and non-specific ChE. In these sections, *specific ChE* was detected in its normal sites, viz., in nerve-fibres about the secretory tubules of the sweat glands, about the digital arteriovenous anastomoses, in red blood cells and in many free endings supplying the volar skin. *Non-specific ChE* was visualized in Meissner tactile corpuscles. No Pacinian corpuscles were visualized in any sections of these biopsies. In sharp contrast with the above findings on the control, water-treated skin, however, were those on the skin from the DFP-treated toes, in which there was apparently complete absence of cholinesterase, both specific and non-specific, at all of the usual sites of localization throughout the sections at the earlier (1 and 2 hour) incubation times. However, after 4 hours incubation, small amounts of the enzymes were perceptible at most of the usual sites.

DISCUSSION

It has long been known that percutaneous absorption through volar skin is considerably less than that which occurs in other skin areas (6). This is believed to be due primarily to the absence of hair follicles and sebaceous glands on the palms and soles. Hence in order to insure the absorption and adequate enzymatic inhibitory effect of the DFP in these experiments, we employed it undiluted, although over a restricted surface area. A satisfactory result was achieved since there was indeed a local inhibition of cholinesterases but no detectable systemic effects.

The histochemical studies clearly indicate the inhibitory effect of ChE's of DFP applied to the volar skin. This is further substantiated by the fact that eccrine sweating was visible on this side and not on the skin of the opposite toe where distilled water was applied. Eccrine sweat glands have a cholinergic innervation and accordingly have specific ChE in the nerve-fibers supplying them. The appearance of sweating on the DFP-treated toes indicates that the specific ChE activity of the nerve-fibers about the volar sweat glands was suppressed, allowing acetylcholine to accumulate and continue to stimulate eccrine sweating. Furthermore, since non-specific ChE is even more sensitive to the inhibitory effect of DFP than is specific ChE (7), the fact that the latter enzyme was inhibited should insure that non-specific ChE was blocked. The depth of penetration of the locally applied DFP is also indicated by the effect on the sweat glands, which are found in the mid- and deep dermis.

It should be emphasized that in the testing of sensation in these studies we could not have

detected minor or subtle sensory alterations but only changes of marked magnitude such as to produce hyperesthesia or complete or near complete loss of sensation in the test area. We fully recognize the possibility that a slight change in sensory perception may have occurred and that we would have been unable to detect it. However, it is highly unlikely that significant changes in sensory perception would go undetected even by the simple methods employed here (8). Since the ChE's appeared to be essentially completely inhibited as indicated by the histochemical findings and the appearance of eccrine sweating, had either or both of these enzymes been essential to the transmission of sensory impulses we should have been able to discern a change in sensory perception. We did not test for alterations in other forms of sensation (cold, heat, itch, tickle). It has been demonstrated previously that the perception of itching is uninfluenced by the local application of DFP (0.1% in peanut oil) on intact human skin (9).

Sensory transmission, in all organs, is still rather poorly understood. There is little evidence which indicates that neurohumoral transmission, which presumably occurs in synaptic and neuromuscular transmission, is also operative in the perception of various forms of sensation. The work of Skouby and his associates (10, 11) in which a lowering of the threshold of sensitivity of skin for pain, cold and warmth following the local introduction of acetylcholine and similar esters was demonstrated, might be regarded as evidence in favor of chemical mediation of these forms of sensation. However, it is emphasized that this sensitizing effect is poorly understood, and is not limited to known neurohumoral or chemically related substances. Menthol, for example, will also produce a similar effect on cold and warmth receptors.

While heavy concentrations of non-specific ChE have been demonstrated in specialized cutaneous nerve-endings, our failure to influence significantly, the perception of light touch, pressure and pricking pain after the local inhibition of cholinesterases by diisopropylfluorophosphate indicates that these enzymes have little or no function in the immediate processes concerned with the transmission of cutaneous sensation. It is possible that the non-specific ChE of the specialized cutaneous nerve-endings may serve a nutritional or trophic function, and that its depletion or prolonged inhibition (weeks to months) would produce a marked alteration in cutaneous sensory perception.

SUMMARY

The inhibition of the cholinesterases (specific and non-specific) of human volar skin by diisopropylfluorophosphate was found to produce no remarkable alteration in the perception of light touch, pressure and pricking pain.

Despite the presence of heavy concentrations of non-specific ChE in the specialized cutaneous sensory nerve-endings of this region it is probable that both this enzyme and specific ChE do not function directly in the transmission of cutaneous sensory impulses at least for the above types of sensation. However, it is possible that non-specific ChE may serve a trophic or nutritional role in the maintenance of these special cutaneous end-organs.

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